**Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**IB Practical 4: Separation of Plant Pigments Using Chromatography**

Purpose: To identify plant pigments by separation and isolation of the pigments using **paper chromatography**.

Pre-Lab Questions: Please read the lab and answer the following questions based on the information provided.

1) What is photosynthesis?

2) What are the main plant pigments? What are the functions of these pigment?

3) What are accessory pigments? What are the functions of these pigments?

4) What is chromatography? How do paper chromatography and thin layer chromatography differ?

5) What is the Rf value?

6) How is the Rf value calculated?

7) What does the Rf value allow us to determine?

Background - Photosynthesis:

Plants are primary producers (along with some bacteria and algae) and produce their own food by using the sun’s energy to transform carbon dioxide and water into glucose. In this process of **photosynthesis**, plants convert the sun’s energy into chemical energy that is stored in the bonds of the glucose molecule. This energy fuels the metabolic processes of cells and is essential for life on earth. Glucose is a simple carbohydrate that provides immediate fuel to cells but it is also a building block for more complex carbohydrates stored by living organisms for future use.

For photosynthesis to transform light energy from the sun into chemical energy (bond energy) in plants, the pigment molecules absorb light to power the chemical reactions. **Plant pigments** are macromolecules produced by the plant, and these pigments absorb specified wavelengths of visible light to provide the energy required for photosynthesis. (see diagram below). Chlorophyll is necessary for photosynthesis, but **accessory pigments** such as carotenoids (carotene and xanthophyll) collect and transfer energy to chlorophyll. Although pigments absorb light, the wavelengths of light that are not absorbed by the plant pigments are reflected back to the eye. The reflected wavelengths are the colors we see in observing the plant. (Example: green pigments reflect green light) Plants contain different pigments, and some of the pigments observed include:

♣ chlorophylls a & b (greens)

♣ carotene (orange)

♣ xanthophyll (yellow)



Background - Paper Chromatography:

The process of **chromatography** separates molecules because of the different solubilities of the molecules in a selected solvent. In **paper chromatography**, paper marked with an unknown, such as plant extract, is placed in a developing chamber with a specified solvent. In **thin layer chromatography**, the solid phase is a glass slide with a material adhered to the surface (see related video). The solvent carries the dissolved pigments as it moves up the paper. The pigments are carried at different rates because they are not equally soluble. A pigment that is the most soluble will travel the greatest distance and a pigment that is less soluble will move a shorter distance.



The distance the pigment travels is unique for that pigment in set conditions and is used to identify the pigment. The ratio is the **Rf (retention factor) value**. Standards are available for comparison

Rf equation:

equation

Preparation of the Sample:

1. Take a strip of chromatography paper approximately 18 cm long. Cut one end into a triangle. With a pencil lightly make a line 2 cm from the pointed end of the paper. 

3. Bend the strip of paper at the blunt end and attach it to the small end of the cork with the push pin. Adjust the length of the paper so that when it is inserted into the test tube, it will touch the bottom without curling.



4. Place a ruler over the leaf so that is covers the pencil line on either end. Using a coin, press down firmly and roll along the ruler edge several times to form a definite green line. Allow the green line to dry thoroughly. Use a fresh area of the leaf and repeat several times until the pencil line is covered completely with a narrow green band. Be careful not to smear this green line



Separation of Pigments:

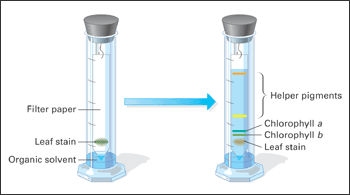
1. Place the test tube in the test tube rack. Using the 6mL syringe, dispense 5 mL of chromatography solvent (isopropanol) in the test tube.

2. Carefully lower the paper strip into the test tube and secure the cork in the top. The solvent must touch the pointed end of the paper but should not touch the green line.

3. Be careful not to slosh the solvent. Allow the tube to stand undisturbed.

4. Observe the solvent movement and the band separation.

5. When the pigments have separated into distinct bands (the solvent has moved approximately half the distance of the paper), lift the cork with paper attached from the test tube. Mark the edge of the solvent front with a pencil. Remove the push pin and detach the paper from the cork. Place the push pin back in the cork and place the cork back on the test tube to minimize fumes. Allow the paper to dry completely.



Analysis and Calculation of Rf values:

****1. On the Student Data Sheet, color the diagram to illustrate the color bands on the chromatogram. Label the band that traveled the greatest distance 1, the next 2, the next 3. Continue until all bands are labeled.

2. Describe the color of each band in Data Table.

3. Measure the distance from the first pencil line to the solvent front. Record this value in Data Table for each pigment.

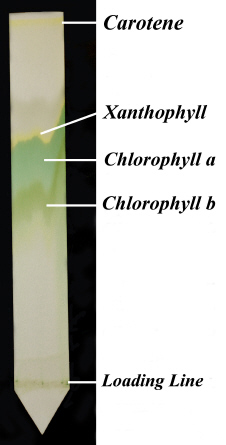
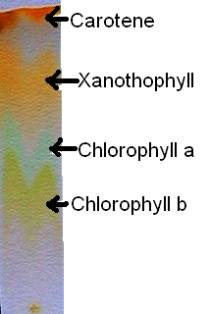
4. Now measure the distance from the first pencil line to the average peak of each color band.

5. Record these values in Data Table. (Depending on the results, groups may have differing numbers of pigments.)

6. Calculate the Rf values for each pigment and record the values in Data Table using the following formula Rf = distance pigment travels/distance the solvent front travels

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| band | Band Color | Distance (mm) | Rf (Calculated) | Pigment |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
| 4 |  |  |  |  |

7. Use the examples below to determine the Rf of other samples and compare to your results to determine the name of each pigment and record the name in Data Table.

Conclusion Questions:

1) Explain why the four pigments moved at different rates through the chromatography paper.

2) Would any leaf from any plant have the pigments that are present in spinach or kale? How would this affect the chromatography with these different leaves?

3) A procedure known as thin layer chromatography would give even better results than chromatography paper. Research and explain the difference between thin layer chromatography and paper chromatography.